

In the claims:

1-43. (Canceled)

44. (Currently Amended) A method of quantifying an amount of at least a first monitor peptide and a second monitor peptide in a biological sample, the first monitor peptide and the second monitor peptide being produced by digestion of a first protein and a second protein, respectively, by a proteolytic agent, the product of the digestion of the biological sample being a digested sample, comprising:

binding contacting the first monitor peptide to sample with (i) a first binding agent, the first binding agent being a polyclonal anti-peptide antibody specific for said first peptide and [[;]]

binding (ii) a known quantity of a labeled version of the said first monitor peptide to the first binding agent, the labeled version of the first monitor peptide being present at a known amount in the digested sample;

binding contacting the second monitor to sample with (i) a second binding agent anti-peptide antibody specific for said second peptide, wherein said the second binding agent being antibody is different than the from said first binding agent antibody and [[;]]

binding (ii) a known quantity of a labeled version of the said second monitor peptide to the second binding agent, the labeled version of the second monitor peptide being present at a known amount in the digested sample;

the first monitor peptide bound to the first binding agent, the labeled version of the first monitor peptide bound to the first binding agent, the second monitor peptide bound to the second binding agent, and the labeled version of the second monitor peptide bound to the second binding agent being bound peptides, peptides produced by the digestion of the biological sample not bound to the first binding agent or the second binding agent being unbound peptides, separating bound peptides bound by said first and said second antibodies from unbound peptides;

eluting said peptides bound by said first and said second antibodies from said antibodies;

measuring the amount of the said first monitor peptide eluted from said first antibody that was separated from unbound peptides using a mass spectrometer;

measuring the amount of the said labeled version of the said first monitor peptide eluted from said first antibody using a mass spectrometer that was separated from unbound peptides;

calculating the amount of the first monitor peptide in the biological sample;

measuring the amount of the said second monitor peptide eluted from said second antibody that was separated from unbound peptides using a mass spectrometer;

measuring the amount of the labeled version of the second monitor peptide eluted from said second antibody that was separated from unbound peptides using a mass spectrometer; and

calculating the amount of the second monitor peptide in the biological sample, wherein said biological sample is a proteolytic digest of a bodily fluid sample.

45-47. (Canceled)

48. (Currently amended) The method of claim 44, wherein at least one of said first and said second antibodies the second binding agent is an is a monoclonal antibody.

49. (Currently amended) The method of claim 44, wherein at least one of said first and said second antibodies the second binding agent is an is a polyclonal monoclonal antibody.

50. (Currently amended) The method of claim 44, wherein the second binding agent is a said first and said second antibodies are both polyclonal antibody antibodies.

51. (Currently amended) The method of claim 44, wherein said first and said second antibodies the second binding agent is an an RNA aptamer are both monoclonal antibodies.

52-53. (Canceled)

54. (Currently amended) The method of claim 44, wherein the labeled version of the first monitor peptide includes at least one site at which a stable isotope is substituted for the corresponding predominant natural isotope in more than 98% of peptide molecules.

55. (Currently amended) The method of claim 44, further comprising:
attaching the first binding agent antibody to a support.

56. (Currently amended) The method of claim 44, further comprising:
attaching the first binding agent antibody to a packed column.

57. (Currently amended) The method of claim 44, further comprising:
attaching the first binding agent antibody to a monolithic porous support.

58. (Currently amended) The method of claim 44, further comprising:
attaching the first binding agent antibody to a mesh.

59. (Currently amended) The method of claim 44, further comprising:
attaching the first binding agent antibody to magnetic beads.

60. (Currently amended) The method of claim 44, wherein the first monitor peptide and the second monitor peptide are selected for optimal differential detection from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer.

61. (Currently amended) A method for quantifying the amount of a monitor peptide produced by digestion of a biological sample by a proteolytic agent, the product of the digestion of the biological sample being a digested sample, comprising:

binding the monitor peptide to a polyclonal antibody;
contacting the sample with

(i) an anti-peptide antibody specific for said peptide;

~~binding a labeled version of the monitor peptide to the polyclonal antibody, the labeled version of the monitor peptide being present at a known amount in the digested sample;~~

(ii) a known quantity of a the monitor peptide bound to the polyclonal antibody and the labeled version of the monitor peptide bound to the polyclonal antibody being bound peptides, peptides produced by digestion of the biological sample and not bound to the polyclonal antibody being unbound peptides,

~~separating bound peptides **bound by said antibody** from unbound peptides~~

eluting said peptide bound by said antibody from said antibody;

~~measuring the relative amounts **amount** of the monitor peptide **eluted from said antibody** separated from unbound peptides and the labeled version of the monitor peptide separated from the unbound peptides using a mass spectrometer; and~~

~~calculating the amount of the monitor peptide in the biological sample;~~

wherein said biological sample is a proteolytic digest of a bodily fluid.

62-63. (Canceled)

64. (Currently amended) The method of claim 61, further comprising:
preparing the labeled version of the monitor peptide.

65. (Currently amended) The method of claim 61, wherein the labeled version of the monitor peptide includes **at least one site at which** a stable isotope **is substituted for the predominant natural isotope in more than 98% of peptide molecules.**

66-70. (Canceled)

71. (Previously presented) The method of claim 67, further comprising:
preparing the labeled version of the monitor peptide.

72. (Previously presented) The method of claim 67, wherein the labeled version of the monitor peptide includes a stable isotope.

73. (Currently amended) The method of claim 44, wherein the separating ~~causes unbound peptides having a total mass to be separated from bound peptides having a total mass, the total mass of~~ from unbound peptides separated from bound peptides being about 100 fold greater than the total mass of bound peptides increases the relative concentration of the bound peptides to unbound peptides by at least 100 fold.

74. (Currently amended) The method of claim 44, wherein said first anti-peptide antibody the first binding agent is created using the first monitor said first peptide or a non-materially modified version of the first monitor peptide.

75. (Currently amended) The method of claim 44, further comprising: creating the first antibody binding agent using the first monitor peptide or a non-materially modified version of the first monitor peptide.

76. (Currently amended) The method of claim 61, wherein the separating ~~causes unbound peptides having a total mass to be separated from bound peptides having a total mass, the total mass of unbound peptides separated from bound peptides being about~~ increases the relative concentration of the bound peptides to unbound peptides by at least 100 fold greater than the total mass of bound peptides.

77. (Currently amended) The method of claim 61, further comprising: creating the polyclonal anti-peptide antibody using the monitor peptide or a non-materially modified version of the monitor peptide.

78. (Currently amended) The method of claim [[67]] 44, wherein the separating ~~causes unbound peptides having a total mass to be separated from~~ said bound peptides having a

~~total mass, the total mass of unbound peptides separated from bound peptides being about 100 fold greater than the total mass of bound peptides~~ **are subjected to a concentrating step after elution from said antibodies and before introduction into said mass spectrometer.**

79-80. (Canceled)

81. (New) The method of claim 61, wherein said bound peptides are subjected to a concentrating step after elution from said antibody and before introduction into said mass spectrometer.

82. (New) The method of claim 61, wherein the anti-peptide antibody is a polyclonal antibody.

83. (New) The method of claim 61, wherein the anti-peptide antibody is a monoclonal antibody.

84. (New) The method of claim 44 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

85. (New) The method of claim 61 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

86. (New) The method of claim 61, wherein the polyclonal antibody is created using the monitor peptide or a non-materially modified version of the monitor peptide.